SHELLFISH TOXINS IN FOOD

A Toxicological Review and Risk Assessment

TECHNICAL REPORT SERIES NO. 14

AUSTRALIA NEW ZEALAND FOOD AUTHORITY
November 2001

ISBN 0 642 34573 2
ISSN 1446-4977
Published November 2001

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SUMMARY

Microscopic unicellular algae (mostly 20 to 200 mm size) form an important component of the plankton diet of shellfish such as mussels, oysters and scallops. Under favourable environmental conditions of light, temperature, salinity, water column stability and nutrients, algal populations of only a few cells can quickly multiply into dense blooms containing millions of cells per litre which can discolour the seawater. Of the estimated 2000 living dinoflagellate species, about 30 species produce toxins that can cause human illness from shellfish or fish poisoning. When humans eat seafood contaminated by these microalgae, they may suffer a variety of gastro-intestinal and neurological illnesses. The most common shellfish poisonings are paralytic shellfish poisoning (PSP) which in extreme cases can lead to death through respiratory paralysis, diarrhetic shellfish poisoning (DSP) which causes severe gastro-intestinal problems and can promote stomach tumours, neurotoxic shellfish poisoning (NSP) which causes respiratory distress, and amnesic shellfish poisoning (ASP) which can lead to permanent brain damage (short-term memory loss).

Poisonous seafood neither looks nor tastes different from uncontaminated seafood, and cooking and other treatments of shellfish do not destroy the toxins. Shellfish and finfish farming areas infested by toxic algal species therefore need to run costly monitoring programmes to check for toxic algae in the water and, whenever these are present, regular tests for toxins in associated seafood products need to be carried out.

Clinical symptoms of toxicity

Biotoxin	**Symptoms**		**Treatment**
	Mild Case	***Extreme Case***	
Paralytic Shellfish Poisoning (PSP)	Within 30 min: tingling sensation or numbness around lips, gradually spreading to face and neck; prickly sensation in fingertips and toes; headache, dizziness, nausea, vomiting, diarrhoea.	Muscular paralysis; pronounced respiratory difficulty; choking sensation; death through respiratory paralysis may occur within 2–24 hrs after ingestion.	Patient has stomach pumped and is given artificial respiration. No lasting effects
Diarrhetic Shellfish Poisoning (DSP)	After 30 min to a few hrs (seldom more than 12 hrs): diarrhoea, nausea, vomiting, abdominal pain.	Chronic exposure may promote tumor formation in the digestive system.	Recovery after 3 days, irrespective of medical treatment.
Amnesic Shellfish Poisoning (ASP)	After 3–5 hrs: nausea, vomiting, diarrhoea, abdominal cramps.	Decreased reaction to deep pain; dizziness, hallucinations, confusion; short-term memory loss; seizures.	

Neurotoxic Shellfish Poisoning (NSP)	After 3-6 hrs: chills, headache, diarrhoea; muscle weakness, muscle and joint pain; nausea and vomiting	Paraesthesia; altered perception of hot and cold; difficulty in breathing, double vision, trouble in talking and swallowing	

Risk characterisation

The serious and in some cases long-term nature of the toxicity associated with seafood toxins makes them a particularly important public health issue. However, there is still a very poor understanding of the target organs for toxicity and of the nature of any dose-response relationship associated with this toxicity. For these reasons, it is still difficult to identify a safe level of exposure to the respective toxins and, therefore, to provide an estimate of the margin of safety at various levels of exposure. Estimates of toxic dose levels have been made at times of algal blooms but it is difficult to get accurate estimates from this data. An acceptable daily intake (ADI) has not been established for any of the seafood toxins.

Dietary exposure estimates for shellfish toxins cannot be conducted in the same way as for other contaminants in food because of the sporadic nature of the contamination, with significant temporal and regional variation in the level of contamination. For the majority of samples tested, the levels of toxins are either zero or very low unless there is an algal bloom when the levels rise dramatically. The use of a overall mean or median contaminant level of toxin to determine normal consumption levels of toxin is therefore of little value.

For PSP, the available data suggests that moderate symptoms of toxicity can occur at intake levels of 120mg of saxitoxin. At the current regulatory level of 80 mg/100g of edible shellfish flesh, this level could be reached after consuming 150g of contaminated shellfish. The margin of safety in this case, therefore, is very small.

The data available suggest that there is a potential for significant health risk from consumption of shellfish contaminated with PSP, ASP, DSP or NSP and that the level of contamination should be kept as low as reasonably achievable. Further information on the individual toxins and the dose-response relationships with the major toxic endpoints will be necessary to further characterise the risk associated with shellfish toxins.

SHELLFISH TOXINS IN FOOD

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INTRODUCTION

Microscopic unicellular algae (mostly 20 to 200 mm size) form an important component of the plankton diet of shellfish such as mussels, oysters and scallops. Under favourable environmental conditions of light, temperature, salinity, water column stability and nutrients, algal populations of only a few cells can quickly multiply into dense blooms containing millions of cells per litre which can discolour the seawater. Of the estimated 2000 living dinoflagellate species, about 30 species produce toxins that can cause human illness from shellfish or fish poisoning. When humans eat seafood contaminated by these microalgae, they may suffer a variety of gastro-intestinal and neurological illnesses. The most common shellfish poisoning is paralytic shellfish poisoning (PSP) which in extreme cases can lead to death through respiratory paralysis, diarrhetic shellfish poisoning (DSP) which causes severe gastro-intestinal problems and can promote stomach tumours, neurotoxic shellfish poisoning (NSP) which causes respiratory distress, and amnesic shellfish poisoning (ASP) which can lead to permanent brain damage (short-term memory loss).

While toxic algal blooms, in a strict sense, are completely natural phenomena which have occurred throughout recorded history, in the past two decades the public health and economic impacts of such events appear to have increased in frequency, intensity and geographic distribution — both on a global scale (Hallegraeff 1993) and within the Australasian region (Hallegraeff 1992). The Australian seafood industry since the mid 1980s and the New Zealand seafood industry since 1993 have become aware of the potential dangers of contamination of cultured and wild shellfish with algal biotoxins.

Poisonous seafood neither looks nor tastes different from uncontaminated seafood, and cooking and other treatments of shellfish do not destroy the toxins. Shellfish and finfish farming areas infested by toxic algal species therefore need to run costly monitoring programmes to check for toxic algae in the water and, whenever these are present, regular tests for toxins in associated seafood products need to be carried out.

Each of the four major groups of toxins encountered through consumption of shellfish (PSP, DSP, ASP and NSP) is discussed below.

PARALYTIC SHELLFISH POISONS

Causative organisms

Potentially toxic PSP dinoflagellates in Australian waters include *Alexandrium (Gonyaulax) catenella* (Port Phillip Bay, South Australia, New South Wales), *Alexandrium minutum* (Port River, South Australia; Western Australia; Shoalhaven, NSW), *Alexandrium tamarense* (presumed toxic strains in Port Phillip Bay), *Gymnodinium catenatum* (Tasmania, Victoria, South Australia, New South Wales) and *Pyrodinium bahamense* var. *compressum* (potential for blooms in the Gulf of Carpentaria) (Hallegraeff 1991, Hallegraeff et al. 1991).

Toxic *Alexandrium* in New Zealand include *A. angustitabulatum, A. catenella, A. minutum, A. ostenfeldii* (some strains toxic) and *A. tamarense* (MacKenzie et al. 1996, Chang et al.

1997). Globally, PSP is responsible for some 2000 cases of human poisoning per year (15% mortality), ranging from temperate waters of Europe, North America and Japan, to the Southern Hemisphere in South Africa, Australia, India, New Zealand, Thailand, Brunei, Sabah, the Philippines and Papua New Guinea (Hallegraeff 1993).

Toxin chemistry

The first PSP toxin to be chemically characterised was named saxitoxin after the butterclam *Saxidomus* from which it was isolated. Since then, at least 20 other toxin fractions have been identified from plankton and shellfish. These toxins all resemble the parent molecule saxitoxin but differ in the type and localisation of derivation. PSP toxins fall into three groups as shown in Table 2.

Table 2. PSP toxins

Carbamate toxins	Sulphamate toxins	Decarbamoyl gonyautoxins
Saxitoxin Neosaxitoxin Gonyautoxins 1,2,3,4	Gonyautoxins 5,6 Fractions C_1,C_2,C_3,C_4	Unnamed

These different PSP toxin fractions show widely different toxic potencies when injected intraperitoneally into mice, ranging from 2045 MU/µmole (saxitoxin) to 16 MU/µmole (fraction C1). One mouse unit (1MU) is the amount of toxin required to kill a mouse weighing 20 g in 15 min upon intraperitoneal injection.

The available methods of detection of PSP are given in Table 3.

Symptoms of human poisoning

PSP toxins block the sodium channels of excitable membranes of the nervous system and associated muscles, inhibiting action potentials and nerve transmission impulses. In vertebrates, the peripheral nervous system is particularly affected; typical symptoms of poisoning include tingling and numbness of the extremities, progressing to muscular incoordination, respiratory distress and muscular paralysis leading to death by asphyxiation in extreme cases. Globally, PSP is responsible for some 2000 cases of human poisoning per year (15% mortality), ranging from temperate waters of Europe, North America and Japan, to the Southern Hemisphere in South Africa, Australia, India, New Zealand, Thailand, Brunei, Sabah, the Phillipines and Papua New Guinea (Hallegraeff 1993).

Dose-response relationship

In humans, 120 to 180 µg PSP can produce moderate symptoms, 400 to 1060 µg PSP can cause death, but 2,000 to 10,000 µg is more likely to constitute a fatal dose. The toxicity of PSP-containing shellfish to humans depends not only on the abundance and toxic potency of the dinoflagellates being filtered, but also on the chemical transformations of the various toxins, either by the shellfish or during food storage and food processing or during digestion in humans.

The sulphamate toxins themselves present a relatively low health risk to humans, but these toxins can easily be transformed under mild acidic conditions to the corresponding carbamate

toxins with a concomitant 10 to 100-fold increase in toxicity. For this reason, the C_{1-4} toxins are often referred to as cryptic PSP toxins. While these conversions are easily accomplished under laboratory conditions, whether these conversions may also occur *in vivo* in the human stomach remains unknown.

Table 3. Detection methods for PSP

Mouse bioassay	To date, the AOAC mouse bioassay (AOAC 1990) is the only internationally, legally accepted method for PSP toxins. In this bioassay, 100 g of shellfish meat is macerated in a blender, gently boiled for 5 min with 100 ml 0.1 N HCl, and 1 ml of the clarified extract (pH adjusted to 2.0-4.0) injected intraperitoneally into a 20 g test mouse. The toxicity of the solution is established by measuring the time from injection to the mouse's last gasping breath, using a table of dose/death time relationships and correcting for the precise weight of the test animal. Substantial errors can result in estimating dose at long or short death times, and sample extracts therefore need to be diluted by trial and error to achieve death times in the range of 5-7 min. Test results can be expressed as mouse units (MU) or can be calibrated against pure saxitoxin and expressed as mg saxitoxin equivalents per 100 g of shellfish meat. The method is relatively easy to perform and requires no special equipment. The major disadvantage is its poor precision ($\pm$ 20%) and insensitivity (detection limit is 50 g saxitoxin/100 g).
HPLC with fluorometic detection	Alternative detection have been developed in recent years. The most successful methods involve the alkaline oxidation of PSP toxins to fluorescent derivatives using periodic acid in sodium phosphate buffer, their separation by high performance liquid chromatography (HPLC) and detection by fluorometry. The HPLC methods developed by Sullivan et al. (1985) and Oshima et al. (1989) have found widespread following. The first method uses a polymer PRP column and gradient elution to separate the 10 most common PSP toxins in a single 20 min run. The second method uses a C8 bonded silica gel column and isocratic elution to separate all known 20 or so PSP toxin fractions in three separate chromatographic runs for sulphamate toxins, gonyautoxins and saxitoxins, respectively. Shellfish with simple toxin profiles (e.g. from *A. minutum*) can be adequately analysed with the Sullivan method, whereas complex toxin profiles (e.g. from *G. catenatum*) can be resolved only with the Oshima method. HPLC methods offer increased sensitivity (10-20 g /100 g) and increased precision (5-10%) compared to mouse bioassays, and during red tide outbreaks can operate continuously with automated injection systems. These chemical methods still require extensive calibration against mouse bioassays before they can become legally accepted.
Emerging assay methods	A mouse neuroblastoma cell bioassay kit for PSP (limit of sensitivity is 2 µg / 100 g) is being developed by Jellett Biotek Ltd (Jellett et al. 1992). However, the limited shelf life of such cell-based assays (1-3 weeks) and false positive results due to interfering substances can pose problems. An ELISA test kit for PSP has also been developed. Radioreceptor binding assays for PSP (Doucette et al.1991) and a method based on the saxitoxin binding protein saxiphilin (Negri and Llewellyn 1998) are also being developed.

Levels in food

The results of the NZ marine biotoxin-monitoring programme are shown in Table 4.

Table 4. PSP results in New Zealand (Sept. 1994 - July 1996)

Biotoxin	Results exceeding regulatory levels (N)	% Total results	Median result exceeding regulatory levels*	Max. levels in shellfish*	No. of biotoxin events
PSP	114/22333	0.5%	88.8	920 (mussel)	11

* µg of saxitoxin equivalent per 100g of edible shellfish equivalent.

The results of monitoring for PSP in Port Phillip Bay and Western Port Bay in Victoria are shown in Table 5.

Table 5. PSP results in mussels in Victoria

Year	No. of Samples	No. of +ve samples	Average level (all samples) (µg/100 g)	Highest level (µg/100g)
1987	11	0	0	0
1988	81	17	29.1	480
1989	88	3	2	66
1990	87	3	3.16	121
1991	34	5	15.4	185
1992	46	25	710.4	10009.6
1993	160	41	64.3	4127.7
1994	188	25	26.6	1286.8
1995	165	10	6.6	406.6
1996	161	0	0	0
1997	44	0	0	0

Extensive testing for PSP has also taken place in Tasmania in mussels, oysters and scallops. The results are shown in Table 6.

Table 6. PSP results in Tasmania

Food	No. of samples	Average level (ug/100 g)	Range
Mussels	168	636	35-18429
Oysters	75	123	38-699
Scallops	6	60	56-83

DIARRHETIC SHELLFISH POISONS

Causative organisms

Potentially toxic DSP plankton dinoflagellates in Australian waters include the planktonic species *Dinophysis acuminata, D.caudata, D. fortii, D.hastata , D. mitra, D. rotundata, D. tripos* and the benthic dinoflagellates *Prorocentrum lima, P. elegans, P. hoffmannianum* and *P.concavum* (Morton & Tindall 1995). These species are omnipresent but their toxicity is variable and unpredictable. Dense blooms (e.g. in Tasmanian and New Zealand waters) can sometimes be completely non-toxic, but at other times shellfish can become toxic even when only sparse dinoflagellate populations are present. In New Zealand, there have been two small outbreaks of DSP.

Toxin chemistry

The causative DSP toxins are fat-soluble polyether compounds. The first toxin to be characterised chemically was okadaic acid (OA), first isolated from the sponge *Halichondria okadai* but now thought to have been derived from associated *P. lima*. Subsequent research revealed the presence in shellfish of okadaic acid derivatives, termed dinophysis toxins (DTX), pectenotoxins (PTX, after the scallop genus *Pecten)* and yessotoxins (YTX, after the scallop *Patinopecten yessoensis)* (Lee et al. 1989). In recent years, a large number of new DSP toxins have been recognised, including DTX2 and an isomer from Irish mussels, a new toxin KT3 (now named spiramino acid) from Irish mussels, pectenotoxin 2 from *D. fortii* in the Adriatic Sea, yessotoxin and analogues from *Gonyaulax polyedra* and *Protoceratium reticulatum.* While yessotoxin has a high intraperitoneal toxicity, its oral potency is very low, and this compound probably needs to be declassified as a DSP toxin (Yasumoto 1997). Diarrhegenic effects have only been proven for OA and DTX1 and DTX3, whereas PTX1-4 causes liver necrosis and YTX damages cardiac muscle after intraperitoneal injection into mice. Some diarrhegenic toxins (OA and DTX1) are potent inhibitors of protein phosphatases and this mode of action may be linked to the observed diarrhoea, degenerative changes in absorptive epithelium of the small intestine, and to tumour promotion.

The currently available methods of detection of DSP are given in Table 7.

Symptoms of human poisoning

Severe vomiting, nausea and diarrhoea symptoms in human shellfish consumers were first recorded in the Netherlands in the 1960s and in Japan in the late 1970s (Kat 1985, Yasumoto et al, 1980). Since then similar problems have been recognised in Spain, France, Scandinavia, Thailand, Chile, Canada and New Zealand. The clinical symptoms of diarrhetic shellfish poisoning (DSP) often may have been mistaken for those of bacterial gastric infections and the problem may be much more widespread than currently thought. Unlike PSP, no human fatalities have been reported and patients usually recover within 3 days. However, some of the toxins involved could act as stomach tumour promoters and thus produce chronic problems in shellfish consumers (Suganuma et al. 1988).

A pipi shellfish-poisoning event (56 patients), which occurred in New South Wales in Dec. 1997, has been circumstantially linked to lipid soluble toxins (a new derivative of okadaic acid; M.Quilliam, in progress) presumably from *D. acuminata* and *D.caudata*, which were abundant in shellfish stomachs (Hallegraeff, unpublished). Low concentrations of DSP toxins (generally < 0.5 mg OA /g) have also been reported from New Zealand shellfish (Jasperse 1993).

Levels in food

The results of the NZ marine biotoxin-monitoring programme are shown in Table 8.

Table7. Detection methods for DSP

Intraperitoneal mouse bioassay	An intraperitoneal mouse bioassay procedure developed by the Japanese Ministry of Health and Welfare (1981) has proved to be susceptible to producing false positive results due to the presence of contaminating free fatty acids in shellfish digestive glands at certain times of the year. This method has now been generally abandoned.
Feeding rat bioassay	A feeding method with white rats has been used successfully in the Netherlands (Kat 1985). Shellfish digestive glands (*not* the complete shellfish meat as in PSP) are mixed with normal rat feed and offered to rats that have been starved for 24 h. After a 16 h observation period, an estimate of DSP toxicity is made on the basis of signs of diarrhea, the consistency of the faeces, and feed refusal. While the intraperitoneal mouse bioassay estimates total toxicity due to a range of lipophilic compounds, the oral dosage rat bioassay evaluates the diarrhetic effect of only certain DSP toxins.
HPLC method	An HPLC method developed for separating DSP toxins by Lee et al. (1987) has greatly advanced this field of research. Shellfish digestive glands are homogenised and extracted with 80% methanol in water, the DSP toxins esterified to fluorescent esters with 9-anthryldiazomethane (ADAM), and the toxins then separated by HPLC and monitored by fluorometry. Problems with the implementation of this method on a routine basis have been noted, resulting from the poor stability of the ADAM reagent and the presence of co-extractives in shellfish tissues, necessitating a silica column cleanup following the derivatization step (Quilliam 1995) .Wild Tasmanian mussels, contaminated by mixed blooms of *D. acuminata* and *D.fortii*, thus proved to contain okadaic acid, dinophysis toxin-1 and an uncharacterised fraction.
Immunoassay test	Semi-quantitative immunoassay test kits for quick detection of OA and / or DTX1 are available from UBE Industries, Tokyo, Japan, and Rougier Bio-Tech., Montreal. While these chemical and immunological techniques represent an important step forward, they are still not sufficiently reliable as a routine method to accurately detect the full range of toxins involved.
Radioactive and colorimetic assays	OA and DTX1 have been identified as inhibitors of protein phosphatases, and this property is now being explored in radioactive and colorimetric assays for their detection. The primary toxins produced by the dinoflagellates are water soluble DTX4 or derivatives, which cannot be detected by ADAM LC procedures. Furthermore, the discovery of an esterase in the outer wall of dinoflagellates led to the recognition that erratic hydrolyses during handling and extraction of shellfish and plankton can lead to erratic results (Quilliam 1998). No routine DSP assays are being carried out in Australia, but testing is conducted at Cawthron Institute and Institute of Environmental Science & Research Ltd (ESR) in New Zealand. Positive mouse bioassays (death in 6 hrs) are followed by ELISA tests for okadaic acid and derivatives, and dilutions of the acetone extract are further examined for the confounding presence of NSP toxins (mouse deaths < 6 hrs). The Asia Pacific Economic Cooperation (APEC) has established the principle of performance based criteria for regulatory purposes. That is, the ability to reliably determine whether the OA series of DSP toxins are present below or above the regulatory level of 20 mg OA equivalent /100 g is the ultimate criterion for choice of a particular analytical method.

Table 8. DSP results in New Zealand (Sept. 1994 - July 1996)

Biotoxin	Results exceeding regulatory levels (N)	% Total results	Median result exceeding regulatory levels*	Max. levels in shellfish*	No. of biotoxin events
DSP	69/10524	0.7%	45 (mussel)	96 (mussel)	9

* µg okadaic acid/100g edible shellfish flesh

AMNESIC SHELLFISH POISONS

Causative organisms

This compound was eventually shown (Bates et al. 1989, Wright et al. 1989) to have derived from the chain-forming diatom *Pseudo-nitzschia multiseries (= P. pungens* f. *multiseries =Nitzschia pungens* f. *multiseries;* Fig. 3i,j). Until this discovery, diatoms were not regarded as sources of toxins. Since then other *Pseudo-nitzschia* species shown to be toxic include *Pseudo-nitzschia australis* (= *Nitzschia pseudoseriata*; in California) and *P. pseudodelicatissima* (in the Bay of Fundy, Canada), while *P. seriata* and *P.delicatissima* have been found to be toxic in laboratory conditions but have not yet been related to domoic acid contamination in the field (Fritz et al. 1992, Lundholm et al. 1994, Martin et al. 1990) . Toxigenic *Pseudo-nitzschia* blooms are also known from the northwest of the US, Japan and New Zealand, Spain and Portugal. Seabird mortalities have been related to consumption of domoic acid contaminated fish in California and Mexico. In addition to bivalve shellfish, razor clams as well as the hepatopancreas and viscera of dungeness crab (US) have also been found to be contaminated. To date, low concentrations of *P. multiseries* have been detected in NSW estuaries, but its toxicity has not yet been confirmed (Hallegraeff 1994). Blooms of *P. pseudodelicatissima* are common in Tasmanian and Victorian coastal waters, but all cultured strains as well as field samples have proved to be nontoxic. *P.australis* is common in New Zealand waters where it has been linked to domoic acid contamination of scallops and greenshell mussels. Toxigenic strains of *P. turgidula* and *P. pungens* have also been reported from New Zealand (Rhodes et al. 1996).

Toxin chemistry

The causative compound, domoic acid, is an excitatory amino acid acting as a glutamate antagonist on the kainate receptors of the central nervous system. The available methods of detection of ASP are given in Table 9.

Human poisoning cases

A serious outbreak of shellfish poisoning occurred in eastern Canada in 1987. The memory loss associated with extreme cases of human intoxication led to the description of the syndrome as amnesic shellfish poisoning (ASP). A limited number of human mortalities have also been associated with ASP in Canada, with immunodepressed patients being most at risk. Humans affected had consumed mussels containing 300-1200 µg/g of domoic acid.

To date the only positive detection of domoic acid in Australian shellfish refers to scallop viscera from Lakes Entrance, Victoria (August 1993) (one sample 26 µg/g; all others <20 µg /g) but the causative organism was not identified in that case (Sang et al. 1992; Arnott 1998). As a precautionary measure, the then Victorian Department of Health and Community Services forbid the sale or supply of scallops other than those that had the viscera removed. Maximum levels of domoic acid detected in New Zealand mussels have been up to 187 µg/g (Marlborough Sounds, Dec. 1994) with scallop digestive glands containing up to 600 µg/g (Jasperse 1993, Rhodes et al. 1996). There have been no poisoning outbreaks in New Zealand.

Table 9. Detection methods for ASP

Mouse bioassay	During the early days of the Canadian crisis, domoic acid was extracted from shellfish using the standardized extraction procedure for mouse bioassay of PSP toxins (Lawrence et al. 1989) but with longer observation times (up to 4 hrs). At toxin levels >40 mg/g domoic acid , mice exhibit characteristic scratching symptoms but this bioassay method is now generally considered not sensitive enough to accurately estimate the proposed quarantine level in Canada of 20 mg/g tissue (AOAC 1991) .
HPLC assay	HPLC is now the preferred analytical technique for the determination of domoic acid in shellfish (Lawrence et al. 1989, Pocklington et al. 1990). A very sensitive procedure, based on reaction with 9-fluorenylmethylchloroformate to form the fluorenylmethoxycarbonyl (FMOC) derivative and HPLC analysis with fluorescence detection, has been developed for monitoring of domoic acid in marine matrices such as seawater and phytoplankton (Pocklington et al. 1990). The detection limit is as low as 15 pg/mL for domoic acid in seawater. This procedure has recently been adapted to shellfish tissue extracts (Quilliam 1995, Quilliam et al. 1995). Domoic acid is extracted from shellfish tissues by homogenization with methanol-water (1:1, v/v). The concentration of domoic acid is determined by HPLC with ultraviolet absorbance detection. Sample extracts are injected following dilution and filtration of the crude extract or after cleanup on strong anion exchange (SAX) solid phase extraction (SPE) cartridges. The latter provides selective isolation of domoic acid and related compounds from interfering substances such as tryptophan, as well as preconcentration to facilitate analysis of trace levels. A photodiode array detector can be used to examine UV spectra in order to confirm domoic acid, but this option may not always be available. The detection limit is 20-30 ng/g (ppb).
Receptor assay	A receptor assay under development for ASP is based on binding to the kainate-glutamate receptor in rat brain synaptosomes, using ^{3}H-kainic acid as a standard (Van Dolah et al. 1994).

Levels in food

The results of the NZ marine biotoxin-monitoring programme are shown in Table 10.

Table 10. ASP results in New Zealand (Sept. 1994 - July 1996)

Biotoxin	Results exceeding regulatory levels (N)	% Total results	Median result exceeding regulatory levels*	Max. levels in shellfish*	No. of biotoxin events
ASP	35/10674	0.3%	45	600 (scallop gut)	8

* µg domoic acid/100g edible shellfish flesh

Extensive routine monitoring has been conducted in Port Phillip Bay, Victoria since 1987 and no domoic acid has been recorded in Bay mussels and scallops. Domoic acid has been detected in scallops in Bass Strait with concentration ranging from 0.12 to 1.2 µg/g in the edible portion. The Victorian Department of Health and Community Services issued an order in 1July 1993 forbidding "the sale or supply of scallops other than scallops which has been opened and from which the viscera had been removed and discarded".

NEUROTOXIC SHELLFISH POISONS

Causative organisms

Until recently, neurotoxic shellfish poisoning (NSP), caused by polyether brevetoxins from the unarmoured dinoflagellate *Gymnodinium breve (=Ptychodiscus brevis),* was considered to be endemic to the Gulf of Mexico and the east coast of Florida. Unexpectedly, in early 1993 more than 180 human shellfish poisonings were reported from New Zealand caused by an organism similar (but not identical) to *Gymnodinium breve* (Jasperse 1993, Haywood et al. 1996). Similar dinoflagellates have also been identified in low concentrations from Victorian, South Australian and West Australian waters. Recent evidence suggests that raphidophyte blooms of *Chattonella marina* (e.g. in Port Lincoln in April 1996; Hallegraeff et al. 1997), and possibly the related genera *Fibrocapsa* and *Heterosigma*, can also produce brevetoxin-like compounds (Kahn et al.1995, 1996, 1997).

Toxin chemistry

Brevetoxins are polyether ladder toxins. Many of these lipid-soluble cyclic polyether compounds have been characterized, but due to the chemical lability of the brevetoxins, analysis of these compounds continues to be problematic (Yasumoto et al. 1989). The New Zealand shellfish toxins (tentatively coded TX1, TX2 and TX3; Satake et al., 1996) differ from well-characterised Florida *G. breve* dinoflagellate toxins (PbTx1-8) in either the side chain, the cyclic ether skeleton or in both.

The available methods of detection of NSP are given in Table 11.

Table 11. Detection methods for NSP

Mouse bioassay	The currently accepted method for the determination of NSP toxins is the American Public Health Association (APHA 1998) procedure based on diethyl-ether extraction of shellfish tissue followed by mouse bioassay. The APHA protocol is widely used in the United States, where the problem of NSP is most acute. After the detection of NSP in New Zealand in 1993, the MAF Regulatory Authority improved the sample preparation method by utilising acetone extraction of lipophilic components, followed by partitioning into dichloromethane (Hannah et al. 1995). Sample extracts are prepared for mouse injection, and the bioassay results are calculated in mouse units .The Hannah procedure is very effective in extracting unknown lipid-soluble toxins from shellfish containing NSP toxins, and the method presents certain advantages compared with the APHA protocol. However, the discovery of a novel bioactive compound (gymnodimine; MacKenzie et al. 1996, Seki et al. 1995), produced by the dinoflagellate *Gymnodinium mikimotoi*, a common species in New Zealand waters during neurotoxic events, has led to the local health authorities to return to the APHA diethyl-ether extraction procedure. Gymnodimine is not extractable by diethyl ether, but it causes very rapid mouse deaths when the dichloromethane procedure is used, while this compound is not considered to present a risk to human health. No testing facilities for NSP are available in Australia, but expertise is available at the Institute of Environmental Science and Research and at AgResearch in New Zealand.
Radioreceptor assay	A sensitive radioreceptor assay for brevetoxin is based on binding to site 5 on the voltage dependent sodium channel in rat brain synaptosomes, using ^{3}H- $PbTx_3$ for quantification (Trainer et al. 1995).
ELISA assay	Neuroblastoma and ELISA assays for NSP are also under development (Garthwaite et al. 1996).

Symptoms of human poisoning

Brevetoxins and their derivatives exert their toxic effect by specific binding to site-5 of voltage-sensitive sodium channels. In humans, the symptoms of NSP intoxication include respiratory distress, as well as eye and nasal membrane irritation, caused principally by exposure to sea-spray aerosols and by direct contact with toxic algal blooms while swimming. No human fatalities from brevetoxin poisoning have ever been reported. The toxins implicated in neurological shellfish poisoning are considered to be primarily ichthyotoxins (fish killing toxins).

Dose-response relationship

Respiratory problems in humans occur at about 10^5-10^6 cells /L, while fish mortality occurs at > 10^6 cells /L.

Levels in food

Levels of NSP during the 1993 New Zealand shellfish poisoning outbreak reached 592 MU / 100 g (Trusewich et al. 1996).

The results of the NZ marine biotoxin-monitoring programme are shown in Table 12.

Table 12. NSP results in New Zealand (Sept. 1994 - July 1996)

Biotoxin	Results exceeding regulatory levels (N)	% Total results	Median result ecxeeding regulatory level*	Max. levels in shellfish*	No. of biotoxin events
NSP (acetone extraction)	162/10479	1.5%	45	945 (oysters)	10
NSP (ether extraction)	17/10485	0.2%	23	26 (various)	10

*mouse units per 100g edible shellfish flesh

In January 1994, mussels from Tamboon Inlet on the Gippsland coast of Victoria contained 27.5 MU / 100 g in association with a *G. breve* type (Arnott 1998). There is no other record of detection of NSP in Australia.

RISK CHARACTERISATION

The serious and in some cases long-term nature of the toxicity associated with seafood toxins makes them a particularly important public health issue. However, there is still a very poor understanding of the target organs for toxicity and of the nature of any dose-response relationship associated with this toxicity. For these reasons, it is still difficult to identify a safe level of exposure to the respective toxins and, therefore, to provide an estimate of the margin of safety at various levels of exposure. Estimates of toxic dose levels have been made at times of algal blooms but it is difficult to get accurate estimates from this data. For none of the seafood toxins has an acceptable daily intake been established.

Dietary exposure estimates for shellfish toxins cannot be conducted in the same way as for other contaminants in food because of the sporadic nature of the contamination, with

significant temporal and regional variation in the level of contamination. For the majority of samples tested, the levels of toxins are either zero or very low unless there is an algal bloom when the levels rise dramatically. The use of a overall mean or median contaminant level of toxin to determine normal consumption levels of toxin is therefore of little value.

For PSP, the available data suggests that moderate symptoms of toxicity can occur at intake levels of 120µg of saxitoxin. At the current regulatory level of 80 µg/100g of edible shellfish flesh, this level could be reached after consuming 150g of contaminated shellfish. The margin of safety in this case, therefore, is very small.

The data available suggest that there is a potential for significant health risk from consumption of shellfish contaminated with PSP, ASP, DSP or NSP and that the level of contamination should be kept as low as reasonably achievable.

ACKNOWLEDGEMENT

ANZFA gratefully acknowledges the assistance of Assoc. Prof. Gustaaf Hallegraeff in preparing the first draft of this report.

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